APPENDIX F

1: Lab Invest. 1998 Aug;78(8):1017-27.

Vascular endothelial growth factor and its receptors in control and diabetic rat eyes.

Gilbert RE, Vranes D, Berka JL, Kelly DJ, Cox A, Wu LL, Stacker SA, Cooper ME.

Department of Medicine, Austin and Repatriation Medical Centre, West Heidelberg, Victoria, Australia.

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific angiogenic and permeability-inducing factor that has been implicated in the pathogenesis of diabetic retinopathy. In the present study, the localization and magnitude of VEGF, VEGF receptor-1 (VEGFR-1), and VEGF receptor-2 (VEGFR-2) gene expression were examined in the eye of streptozotocin-induced diabetic rats using quantitative in situ hybridization. VEGF protein was also examined by immunohistochemistry. Abundant VEGF mRNA and protein were present in the retinae of control rats. In the retinae of diabetic rats, VEGF gene expression was increased compared with control animals ($p = 0.001$). The increase in VEGF mRNA was noted in the ganglion cell layer and inner nuclear layer but not in the pigment epithelium of the retina. VEGF was also detected in blood vessels, ciliary body, and lens epithelium in both control and diabetic rats. The distributions of VEGFR-1 and VEGFR-2 were similar in both control and diabetic rats. VEGFR-1 mRNA was present beneath the inner limiting membrane and in the ganglion cell layer, inner nuclear layer, outer plexiform layer, and outer limiting membrane of the retina; it was also detected in blood vessels, the ciliary body, and the cornea. The magnitude and distribution of ocular VEGFR-1 mRNA were not affected by experimental diabetes. Expression of VEGFR-2 mRNA

was noted in the inner nuclear layer and pigment epithelium of the retina and in blood vessels. An increase in VEGFR-2 mRNA in the diabetic retina was restricted to the inner nuclear layer. The presence of VEGF and its receptors in the control retina suggests a physiologic role for VEGF within the eye. The changes in retinal expression of VEGF and VEGFR-2 in association with diabetes suggest a role for this pathway in diabetic retinopathy.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 9714188 [PubMed - indexed for MEDLINE]

☐ **2:** J Invest Dermatol. 1998 Jul;111(1):1-6.

Related Articles, Links

Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice.

Detmar M, Brown LF, Schön MP, Elicker BM, Velasco P, Richard L, Fukumura D, Monsky W, Claffey KP, Jain RK.

Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA.

Vascular endothelial growth factor (VEGF) has been implicated in the pathologic angiogenesis observed in psoriasis and other chronic inflammatory skin diseases that are characterized by enhanced expression of VEGF by epidermal keratinocytes and of VEGF receptors by tortuous microvessels in the upper dermis. To investigate the functional importance of chronic VEGF overexpression in vivo, we used a keratin 14 promoter expression cassette containing the gene for murine VEGF164 to selectively target VEGF expression to basal epidermal keratinocytes in transgenic mice. These mice demonstrated an increased density of tortuous cutaneous blood capillaries with elevated expression levels of the high affinity VEGF receptors, VEGFR-1 and VEGFR-2, most prominently during the neonatal period. In contrast, no abnormalities of lymphatic vessels were detected. In addition, the number of mast cells in the upper dermis was significantly increased in transgenic skin. Intravital fluorescence microscopy revealed highly increased leukocyte rolling and adhesion in postcapillary skin venules that were both inhibited after injection of blocking antibodies against E- and P-selectin. Combined blocking antibodies against intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 were without effect, whereas an anti-vascular cell adhesion molecule-1/VLA-4 antibody combination almost completely normalized the enhanced leukocyte adhesion in transgenic mice. This study reveals VEGF as a growth factor specific for blood vessels, but not lymphatic vessels, and demonstrates that chronic orthotopic overexpression of VEGF in the epidermis is sufficient to induce cardinal features of chronic skin inflammation,

providing a molecular link between angiogenesis, mast cell accumulation, and leukocyte recruitment to sites of inflammation.

Publication Types:
- Research Support, Non-U.S. Gov't
- Research Support, U.S. Gov't, P.H.S.

PMID: 9665379 [PubMed - indexed for MEDLINE]

3: J Biol Chem. 1998 Apr 3;273(14):8413-8.

Related Articles, Links

Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-C.

Ristimäki A, Narko K, Enholm B, Joukov V, Alitalo K.

Department of Bacteriology and Immunology, the Haartman Institute, and the Department of Obstetrics and Gynecology, Haartmaninkatu 2, FIN-00290 University of Helsinki, Helsinki, Finland.

Vascular endothelial growth factor (VEGF) is a prime regulator of normal and pathological angiogenesis. Three related endothelial cell growth factors, VEGF-B, VEGF-C, and VEGF-D were recently cloned. We have here studied the regulation of VEGF-C, a lymphatic endothelial growth factor, by angiogenic proinflammatory cytokines. Interleukin (IL)-1beta induced a concentration- and a time-dependent increase in VEGF-C, but not in VEGF-B, mRNA steady-state levels in human lung fibroblasts. The increase in VEGF-C mRNA levels was mainly due to increased transcription rather than elevated mRNA stability as detected by the nuclear run-on method and by following mRNA decay in the presence of an inhibitor of transcription, respectively. In contrast, angiopoietin-1 mRNA, encoding the ligand for the endothelial-specific Tek/Tie-2 receptor, was down-regulated by IL-1beta. Tumor necrosis factor-alpha and IL-1alpha also elevated VEGF-C mRNA steady-state levels, whereas the IL-1 receptor antagonist and dexamethasone inhibited the effect of IL-1beta. Experiments with cycloheximide indicated that the effect of IL-1beta was independent of protein synthesis. Hypoxia, which is an important inducer of VEGF expression, had no effect on VEGF-B or VEGF-C mRNA levels. IL-1beta and tumor necrosis factor-alpha also stimulated the production of VEGF-C protein by the fibroblasts. Cytokines and growth factors have previously been shown to down-regulate VEGF receptors in vascular endothelial cells. We found that the mRNA for the VEGF- and VEGF-C-binding VEGFR-2 (KDR/Flk-1) was stimulated by IL-1beta in human umbilical vein endothelial cells, whereas the mRNA levels of VEGFR-1 (Flt-1) and VEGFR-3 (Flt-4) were not altered. Our data suggest that in addition to VEGF,

VEGF-C may also serve as an endothelial stimulus at sites of cytokine activation. In particular, these results raise the possibility that certain proinflammatory cytokines regulate the lymphatic vessels indirectly via VEGF-C.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 9525952 [PubMed - indexed for MEDLINE]

4: Exp Nephrol. 1998 Jan-Feb;6(1):17-21.

Related Articles, Links

KARGER Full Text

Origin of glomerular capillaries: is the verdict in?

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Classical studies with murine embryonic kidneys (metanephroi) grown in organ culture or on the avian chorio-allantoic membrane have suggested that kidney endothelia arise by ingrowth or angiogenesis. More recent studies, however, indicate that glomerular capillaries and arterioles may form in situ by vasculogenesis when more realistic experimental conditions are deployed: these include glomerulogenesis after transplantation of metanephroi to the nephrogenic renal cortex of mice as well as development in oculo. This conclusion is supported by the finding that receptor tyrosine kinases such as VEGFR-1/2 and Tie-1, characteristic of endothelial precursors, are expressed in the metanephros at a stage when no patent vessels are apparent. Further studies are required to determine the origin of endothelial cells in renal vessels of larger calibre.

Publication Types:
- Research Support, Non-U.S. Gov't
- Review

PMID: 9523169 [PubMed - indexed for MEDLINE]

5: Proc Natl Acad Sci U S A. 1998 Jan 20;95(2):548-53.

Related Articles, Links

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Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and

VEGF receptor 3 (Flt4).

Achen MG, Jeltsch M, Kukk E, Mäkinen T, Vitali A, Wilks AF, Alitalo K, Stacker SA.

Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria, Australia. Marc.achen@ludwig.edu.au

We have identified a member of the VEGF family by computer-based homology searching and have designated it VEGF-D. VEGF-D is most closely related to VEGF-C by virtue of the presence of N- and C-terminal extensions that are not found in other VEGF family members. In adult human tissues, VEGF-D mRNA is most abundant in heart, lung, skeletal muscle, colon, and small intestine. Analyses of VEGF-D receptor specificity revealed that VEGF-D is a ligand for both VEGF receptors (VEGFRs) VEGFR-2 (Flk1) and VEGFR-3 (Flt4) and can activate these receptors. However. VEGF-D does not bind to VEGFR-1. Expression of a truncated derivative of VEGF-D demonstrated that the receptor-binding capacities reside in the portion of the molecule that is most closely related in primary structure to other VEGF family members and that corresponds to the mature form of VEGF-C. In addition, VEGF-D is a mitogen for endothelial cells. The structural and functional similarities between VEGF-D and VEGF-C define a subfamily of the VEGFs.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 9435229 [PubMed - indexed for MEDLINE]

PMCID: PMC18457

☐ **6:** Biochem Biophys Res Commun. 1997 Sep 18;238(2):487-91.

Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

The phosphorylated 1169-tyrosine containing region of flt-1 kinase (VEGFR-1) is a major binding site for PLCgamma.

Sawano A, Takahashi T, Yamaguchi S, Shibuya M.

Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, 108, Japan.

Flt-1, a tyrosine kinase receptor for vascular endothelial growth factor (VEGF), plays important roles in the angiogenesis required for embryogenesis and in monocyte/macrophage migration. However, the signal transduction of Flt-1 is poorly understood due to its very weak tyrosine kinase activity. Therefore, we overexpressed Flt-1 in insect

cells using the Baculovirus system in order to examine for autophosphorylation sites and association with adapter molecules such as phospholipase Cgamma-1 (PLCgamma). Tyr-1169 and Tyr-1213 on Flt-1 were found to be auto-phosphorylated, but only a phenylalanine mutant of Tyr-1169 strongly suppressed its association with PLCgamma. In Flt-1 overexpressing NIH3T3 cells, VEGF induced autophosphorylation of Flt-1, tyrosine-phosphorylation of PLCgamma and protein kinase C-dependent activation of MAP kinase. These results strongly suggest that Tyr-1169 on Flt-1 is a major binding site for PLCgamma and important for Flt-1 signal transduction within the cell. Copyright 1997 Academic Press.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 9299537 [PubMed - indexed for MEDLINE]

7: Cancer Res. 1997 Sep 1;57(17):3852-9.

Related Articles, Links

Full Text FREE Cancer Res

Up-regulation of flk-1/vascular endothelial growth factor receptor 2 by its ligand in a cerebral slice culture system.

Kremer C, Breier G, Risau W, Plate KH.

Department of Neuropathology, Neurocenter, Freiburg University Medical School, Germany.

Vascular endothelial growth factor (VEGF) and its tyrosine kinase receptors VEGFR-1 (flt-1) and VEGFR-2 (flk-1/KDR) are key mediators of physiological and pathological angiogenesis. They are expressed in most tissues during embryonic development but are down-regulated in the adult, when angiogenesis ceases. Up-regulation of VEGFR-2 and of VEGF are observed in many pathological conditions under which angiogenesis is reinduced. A major regulator of VEGF expression is hypoxia. Although the temporal expression pattern of VEGFR-2 parallels VEGF expression to a high extent, little is known about its regulation. Here, we show that VEGFR-2 is highly expressed in early postnatal mouse brain but is down-regulated commencing at postnatal day 15 (P15) of mouse brain development and is hardly detectable in P30 mouse brain. Using P30 mouse brain slices, we observed that hypoxia up-regulates VEGFR-2 in the slices but not in human umbilical vein endothelial cells, suggesting the presence of a hypoxia-inducible factor in the murine neuroectoderm that up-regulates VEGFR-2. To identify the factors involved, normoxic P30 cerebral slices were cultured with growth factors that are either hypoxia-inducible (e.g., PDGF-BB, erythropoietin, and VEGF) and/or are known to act on endothelial cells (e.g., PDGF-BB, VEGF, and PIGF). Exogenously added recombinant VEGF led to an up-regulation

of VEGFR-2 expression, which could be inhibited by preincubation with a neutralizing anti-VEGF antibody. Addition of PDGF-BB, PIGF, and erythropoietin had no effect on VEGFR-2 expression. Our results suggest a differential but synergistic regulation by hypoxia of VEGF and VEGFR-2: a direct induction of VEGF that subsequently up-regulates VEGFR-2 in endothelial cells. This autoenhancing system may represent an important mechanism of tumor angiogenesis.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 9288799 [PubMed - indexed for MEDLINE]

8: Blood. 1997 Aug 15;90(4):1365-72.

Related Articles, Links

Tat-human immunodeficiency virus-1 induces human monocyte chemotaxis by activation of vascular endothelial growth factor receptor-1.

Mitola S, Sozzani S, Luini W, Primo L, Borsatti A, Weich H, Bussolino F.

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Human immunodeficiency virus-1 (HIV-1) Tat protein can be released by infected cells and activates mesenchymal cells. Among these, monocytes respond to Tat by migrating into tissues and releasing inflammatory mediators. In the present study, we have examined the molecular mechanism of monocyte activation by Tat, showing that this viral protein signals inside the cells through the tyrosine kinase receptor for vascular endothelial growth factor encoded by fms-like tyrosine kinase gene (VEGFR-1/Flt-1). Subnanomolar concentrations of Tat induced monocyte chemotaxis, which was inhibited by cell preincubation with vascular-endothelial growth factor-A (VEGF-A). This desensitisation was specific for VEGF-A, because it not was observed with FMLP. In addition, the soluble form of VEGFR-1 specifically inhibited polarization and migration induced by Tat and VEGF-A, thus confirming the common use of this receptor. Binding studies performed at equilibrium by using radiolabeled Tat showed that monocytes expressed a unique class of binding site, with a kd of approximately 0.2 nmol/L. The binding of radiolabeled Tat to monocyte surface and the cross-linking to a protein of 150 kD was inhibited specifically by an excess of cold Tat or VEGF-A. Western blot analysis with an antibody anti-VEGFR-1/Flt-1 performed on monocyte phosphoproteins immunoprecipitated by an monoclonal antibody anti-phosphotyrosine showed that Tat induced a rapid phosphorylation in tyrosine residue of the 150-kD VEGFR-1/Flt-1.

Taken together, these results suggest that biologic activities of HIV-1 Tat in human monocytes may, at least in part, be elicited by activation of VEGFR-1/Flt-1.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 9269752 [PubMed - indexed for MEDLINE]

9: Thromb Haemost. 1997 Jul;78(1):678-83.

Related Articles, Links

Angiogenesis in embryos and ischemic diseases.

Breier G, Damert A, Plate KH, Risau W.

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Angiogenic growth factors and their endothelial receptors are thought to function as major regulators of blood vessel formation. Vascular endothelial growth factor (VEGF) and its receptors, Flt-1 (VEGFR-1) and Flk-1 (VEGFR-2), as well as Angiopoietin-1 and its receptor, Tie-2, represent key signal transduction systems involved in the regulation of embryonic vascular development. The expression of these molecules correlates with phases of blood vessel formation during embryogenesis. Inactivation of any of the genes encoding these molecules in mouse embryos results in defective vascular development and embryonic lethality around mid-gestation. In addition, the VEGF signal transduction system has been implicated in the regulation of pathological blood vessel growth during certain angiogenesis-dependent diseases that are often associated with tissue ischemia, such as proliferative retinopathy or solid tumor growth. This hypothesis is substantiated by experiments, in which the inhibition of VEGF signal transduction resulted in the the inhibition of neovascularization in these diseases. Thus, the VEGF signal transduction system represents a useful target for an anti-angiogenic therapy.

Publication Types:
- Research Support, Non-U.S. Gov't
- Review

PMID: 9198238 [PubMed - indexed for MEDLINE]

10: Angiogenesis. 1997;1(1):84-101.

Related Articles, Links

SpringerLink

A molecular and genetic analysis of renalglomerular capillary development.

Loughna S, Hardman P, Landels E, Jussila L, Alitalo K, Woolf AS.

Developmental Biology, Institute of Child Health, London WC1N 1EH, UK.

The adult kidney is highly vascular and receives about 20% of the cardiac output, yet the mode of development of the glomerular capillaries is not fully understood. At the inception of nephrogenesis the condensed metanephric mesenchyme contains no patent capillaries. However, in this current study we detected vascular endothelial growth factor (VEGF) mRNA and protein in uninduced mouse E11 metanephric mesenchyme and in cell lines from this tissue. Moreover, transcripts for receptor tyrosine kinases which are markers of endothelial precursors (VEGFR-1/Flt-1, VEGFR-2/Flk-1 and Tie-1) were expressed by the E11 mesenchyme. In transgenic mice, Tie1/LacZ-expressing cells were identified in E11 renal mesenchyme when patent vessels were absent. Moreover, a similar pattern of transgene expression was detected within intermediate mesoderm condensing to form metanephric mesenchyme. When Tie-1/LacZ E11 metanephroi were transplanted into the nephrogenic cortex of wild-type mice, transgene-expressing capillary loops were detected in glomeruli developing in donor tissue. In contrast, glomerular Tie-1/LacZ-positive vessels never developed in rudiments in organ culture. We postulate that endothelial precursors are present at the inception of the mouse nephrogenesis, and these differentiate and undergo morphogenesis into glomerular capillaries when experimental conditions resemble those found in the metanephros in vivo.

PMID: 14517396 [PubMed - as supplied by publisher]

11: Histol Histopathol. 1996 Oct;11(4):1049-61.

Related Articles, Links

Pathology, genetics and cell biology of hemangioblastomas.

Wizigmann-Voos S, Plate KH.

Neurozentrum der Albert-Ludwigs-Universität, Abteilung Neuropathologie, Freiburg, Germany.

Hemangioblastomas are highly vascularized tumors of not well-defined histological origin which are frequently associated with cysts. They arise preferentially in cerebellum, medulla and spinal cord and are histologically indistinguishable from vascular lesions in the retina (so-called angiomatosis retinae). Hemangioblastomas are the most

frequent manifestations of the von Hippel-Lindau (VHL) disease, an autosomal-dominant inherited cancer syndrome but also occur as sporadic non-hereditary tumors. The VHL tumor suppressor gene has recently been cloned and enormous progress has been made towards the understanding of molecular biology and biological function of the VHL gene. Germline mutations in VHL patients, as well as somatic mutations in different tumors, including hemangioblastomas, have been identified, its ability to act as a tumor suppressor in vivo has been confirmed, and interaction with transcription factors Elongin B and C leading to inhibition of transcriptional elongation has been demonstrated. The mechanism underlying neovascularization and cyst formation in hemangioblastomas and how this is linked to inactivation of the VHL tumor suppressor gene is not known. However, the finding of dramatic up-regulation of vascular endothelial growth factor (VEGF), a potent endothelial cell growth factor with vascular permeability-inducing activity, in stromal cells and the corresponding receptors, VEGFR-1 and VEGFR-2, in tumor endothelial cells suggests that angiogenesis and cyst formation in hemangioblastomas may be regulated by this signaling pathway via a paracrine mechanism.

Publication Types:
- Research Support, Non-U.S. Gov't
- Review

PMID: 8930647 [PubMed - indexed for MEDLINE]

☐ **12:** J Biol Chem. 1996 May 10;271(19):11500-5.

Related Articles, Links

Transforming growth factor beta 1 down-regulates vascular endothelial growth factor receptor 2/flk-1 expression in vascular endothelial cells.

Mandriota SJ, Menoud PA, Pepper MS.

Department of Morphology, University Medical Center, Geneva, Switzerland.

Although the importance of the vascular endothelial growth factor (VEGF)/VEGF tyrosine kinase receptor (VEGFR) system in angiogenesis is well established, very little is known about the regulation of VEGFR expression in vascular endothelial cells. We have cloned partial cDNAs encoding bovine VEGFR-1 (flt) and -2 (flk-1) and used them to study VEGFR expression by bovine microvascular- and large vessel-derived endothelial cells. Both cell lines express flk-1, but not flt. Transforming growth factor beta 1 (TGF-beta 1) reduced the high affinity 125I-VEGF binding capacity of both cell types in a dose-dependent manner, with a 2.0-2.7-fold

decrease at 1-10 ng/ml. Cross-linking experiments revealed a decrease in 125I-VEGF binding to a cell surface monomeric protein corresponding to Flk-1 on the basis of its affinity for VEGF, molecular mass (185-190 kDa), and apparent internalization after VEGF binding. Immunoprecipitation and Western blot experiments demonstrated a decrease in Flk-1 protein expression, and TGF-beta 1 reduced flk-1 mRNA levels in a dose-dependent manner. These results imply that TGF-beta 1 is a major regulator of the VEGF/Flk-1 signal transduction pathway in endothelial cells.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 8626709 [PubMed - indexed for MEDLINE]

13: Am J Pathol. 1996 Mar;148(3):763-75.

Related Articles, Links

Vascular growth factors and receptors in capillary hemangioblastomas and hemangiopericytomas.

Hatva E, Böhling T, Jääskeläinen J, Persico MG, Haltia M, Alitalo K.

Neurobiology Laboratory, University of Helsinki, Finland.

Capillary hemangioblastomas and hemangiopericytomas are highly vascular central nervous system tumors of controversial origin. Of interest in their pathogenesis are mechanisms regulating endothelial cell growth. The endothelial cell mitogen vascular endothelial growth factor (VEGF) stimulates angiogenesis, and together with its two receptor tyrosine kinases VEGFR-1(FLT1) and VEGFR-2(KDR), is up-regulated during the malignant progression of gliomas. We have analyzed the expression of VEGF and its receptors, the related placental growth factor (PlGF) and the endothelial receptors FLT4 and Tie by in situ hybridization in capillary hemangioblastomas and hemangiopericytomas. VEGF mRNA was up-regulated in all of the hemangiopericytomas studied and highly expressed in the stromal cells of hemangioblastomas. In addition, some hemangioblastoma tumor cells expressed high levels of PlGF. Significantly elevated levels of Tie mRNA, Tie protein, VEGFR-1, and VEGFR-2 but not FLT4 mRNAs were observed in the endothelia of both tumor types. In hemangioblastomas, however, the receptors were also highly expressed by a subpopulation of stromal cells. Consistent results were obtained for a human hemangioblastoma cell line in culture. Up-regulation of the endothelial growth factors and receptors may result in autocrine or paracrine stimulation of endothelial cells and their precursors involved in the genesis of these two vascular tumors.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 8774132 [PubMed - indexed for MEDLINE]

PMCID: PMC1861732

14: EMBO J. 1996 Jan 15;15(2):290-98.

Related Articles, Links

Erratum in:
- EMBO J. 1996 Apr 1;15(7):1751.

A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases.

Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, Saksela O, Kalkkinen N, Alitalo K.

Department of Virology, Haartman Institute, University of Helinski, Finland.

Angiogenesis, the sprouting of new blood vessels from pre-existing ones, and the permeability of blood vessels are regulated by vascular endothelial growth factor (VEGF) via its two known receptors Flt1 (VEGFR-1) and KDR/Flk-1 (VEGFR-2). The Flt4 receptor tyrosine kinase is related to the VEGF receptors, but does not bind VEGF and its expression becomes restricted mainly to lymphatic endothelia during development. In this study, we have purified the Flt4 ligand, VEGF-C, and cloned its cDNA from human prostatic carcinoma cells. While VEGF-C is homologous to other members of the VEGF/platelet derived growth factor (PDGF) family, its C-terminal half contains extra cysteine-rich motifs characteristic of a protein component of silk produced by the larval salivary glands of the midge, Chironomus tentans. VEGF-C is proteolytically processed, binds Flt4, which we rename as VEGFR-3 and induces tyrosine autophosphorylation of VEGFR-3 and VEGFR-2. In addition, VEGF-C stimulated the migration of bovine capillary endothelial cells in collagen gel. VEGF-C is thus a novel regulator of endothelia, and its effects may extend beyond the lymphatic system, where Flt4 is expressed.

Publication Types:
- Comparative Study
- Research Support, Non-U.S. Gov't

PMID: 8617204 [PubMed - indexed for MEDLINE]

PMCID: PMC449944

15: Glia. 1995 Nov;15(3):339-47.

Related Articles, Links

Angiogenesis in malignant gliomas.

Plate KH, Risau W.

Neurozentrum, Albert-Ludwigs Universität, Freiburg, Germany.

One event that accompanies glioma progression is the upregulation of angiogenesis. Low-grade gliomas are moderately vascularized tumors whereas high-grade gliomas show prominent microvascular proliferations and areas of high vascular density. To analyze the molecular mechanisms underlying glioma angiogenesis, we studied the expression of vascular endothelial growth factor (VEGF) and its tyrosine kinase receptors VEGFR-1 and VEGFR-2 during normal brain development and glioma-induced angiogenesis. Our results suggest a paracrine control of angiogenesis and endothelial cell proliferation that is tightly regulated and transient in the embryonic brain, switched off in the normal adult brain, and turned on in tumor cells (VEGF) and the host vasculature (VEGFR-1 and -2) during tumor progression. It is unknown how VEGF and VEGF receptors are upregulated during glioma angiogenesis, but there is recent evidence that VEGF as well as endogenous inhibitors of angiogenesis could be under control of the tumor suppressor genes p53 and VHL.

Publication Types:
- Research Support, Non-U.S. Gov't
- Review

PMID: 8586468 [PubMed - indexed for MEDLINE]

16: EMBO J. 1996 Apr 1;15(7):1751.

Related Articles, Links

Erratum for:
- EMBO J. 1996 Jan 15;15(2):290-98.

A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases.

Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, Saksela O, Kalkkinen N, Alitalo K.

Publication Types:
- Published Erratum

PMID: 8612600 [PubMed - indexed for MEDLINE]

PMCID: PMC450088

17: Development. 1996 Dec;122(12):3829-37.

Related Articles, Links

VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development.

Kukk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V, Alitalo K.

Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Finland.

The vascular endothelial growth factor family has recently been expanded by the isolation of two new VEGF-related factors, VEGF-B and VEGF-C. The physiological functions of these factors are largely unknown. Here we report the cloning and characterization of mouse VEGF-C, which is produced as a disulfide-linked dimer of 415 amino acid residue polypeptides, sharing an 85% identity with the human VEGF-C amino acid sequence. The recombinant mouse VEGF-C protein was secreted from transfected cells as VEGFR-3 (Flt4) binding polypeptides of 30-32x10(3) Mr and 22-23x10(3) Mr which preferentially stimulated the autophosphorylation of VEGFR-3 in comparison with VEGFR-2 (KDR). In in situ hybridization, mouse VEGF-C mRNA expression was detected in mesenchymal cells of postimplantation mouse embryos, particularly in the regions where the lymphatic vessels undergo sprouting from embryonic veins, such as the perimetanephric, axillary and jugular regions. In addition, the developing mesenterium, which is rich in lymphatic vessels, showed strong VEGF-C expression. VEGF-C was also highly expressed in adult mouse lung, heart and kidney, where VEGFR-3 was also prominent. The pattern of expression of VEGF-C in relation to its major receptor VEGFR-3 during the sprouting of the lymphatic endothelium in embryos suggests a paracrine mode of action and that one of the functions of VEGF-C may be in the regulation of angiogenesis of the lymphatic vasculature.

Publication Types:
- Comparative Study
- Research Support, Non-U.S. Gov't

PMID: 9012504 [PubMed - indexed for MEDLINE]

18: Cell Tissue Res. 1997 May;288(2):207-23.

Related Articles, Links

SpringerLink

Expression of the avian VEGF receptor homologues Quek1 and Quek2 in blood-vascular and lymphatic endothelial and non-endothelial cells during quail embryonic development.

Wilting J, Eichmann A, Christ B.

Anatomisches Institut II der Albert-Ludwigs-Universität, Albertstrasse 17, D-79104 Freiburg, Germany. wilting@ruf.uni-freiburg.de

We have studied the expression of Quek1 and Quek2 (VEGFR-2 and VEGFR-3, respectively) in quail embryos from day 2 to day 16 by in situ hybridization with digoxigenin-labelled riboprobes on whole-mounts and paraffin sections. Parallel sections were also stained with the QH1 antibody to detect all endothelial cells and with an antibody against alpha-smooth-muscle-actin to reveal the media of blood vessels. Quek1/VEGFR-2 is a marker of blood-vascular and lymphatic endothelial cells throughout development. In 2-day-old embryos, it is expressed in the intra-embryonic vascular plexus, in cells (most probably angioblasts) located in the paraxial head mesoderm and in the somites, and caudo-laterally from Hensen's node. Thereafter, until about day 9, Quek1 is expressed in all endothelial cells. Cells positive and negative for Quek1 can later be found within the same vessel. Quek1 is additionally expressed in lymphatic endothelial cells. Occasionally, some non-endothelial cell types express Quek1. Quek2/VEGFR-3 is also a marker of endothelial cells; however, its expression pattern differs from that of Quek1. In 2-day-old embryos, Quek2 is expressed in the notochord and the intra-embryonic vascular plexus. Whereas all endothelial cells are Quek2-positive in 3-day-old embryos, expression is subsequently reduced to a subset of endothelial cells: arteries become Quek2-negative and then expression of Quek2 is limited to a few vessels that appear to be lymphatic. Endothelial cells of lymph nodes and the periaortal lymphatic vessels are Quek2-positive in later stages. A few non-endothelial cells express Quek2.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 9082957 [PubMed - indexed for MEDLINE]

19: Science. 1997 May 30;276(5317):1423-5.

Related Articles, Links

Science FREE WITH REGISTRATION

Erratum in:
- Science 1997 Jul 25;277(5325):463.

Hyperplasia of lymphatic vessels in VEGF-C transgenic mice.

Jeltsch M, Kaipainen A, Joukov V, Meng X, Lakso M, Rauvala H, Swartz M, Fukumura D, Jain RK, Alitalo K.

Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Finland.

No growth factors specific for the lymphatic vascular system have yet been described. Vascular endothelial growth factor (VEGF) regulates vascular permeability and angiogenesis, but does not promote lymphangiogenesis. Overexpression of VEGF-C, a ligand of the VEGF receptors VEGFR-3 and VEGFR-2, in the skin of transgenic mice resulted in lymphatic, but not vascular, endothelial proliferation and vessel enlargement. Thus, VEGF-C induces selective hyperplasia of the lymphatic vasculature, which is involved in the draining of interstitial fluid and in immune function, inflammation, and tumor metastasis. VEGF-C may play a role in disorders involving the lymphatic system and may be of potential use in therapeutic lymphangiogenesis.

Publication Types:
- Research Support, Non-U.S. Gov't
- Research Support, U.S. Gov't, P.H.S.

PMID: 9162011 [PubMed - indexed for MEDLINE]

☐ **20:** Carcinogenesis. 1997 Jun;18(6):1155-61.

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Over expression of vascular endothelial growth factor and its receptor during the development of estrogen-induced rat pituitary tumors may mediate estrogen-initiated tumor angiogenesis.

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Estrogens, which have been associated with several types of human and animal cancers, can induce tumor angiogenesis in the pituitary of Fischer 344 rats. The mechanistic details of tumor angiogenesis

induction, during estrogen carcinogenesis, are still unknown. To elucidate the role of estrogen in the regulation of tumor angiogenesis in the pituitary of female rats, the density of blood vessels was analysed using factor VIII related antigen (FVIIIRAg) immunohistochemistry and the expression of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) was examined by Western blot and immunohistochemical analysis. The expression of VEGF receptor (VEGFR-2/Flk-1/KDR) was also examined by immunohistochemistry. The results demonstrated that 17beta-estradiol (E2) induces neovascularization, as well as the growth and enlargement of blood vessels after 7 days of exposure. The high tumor angiogenic potential was associated with an elevated VEGF/VPF protein expression in the E2 exposed pituitary of ovariectomized (OVEX) rats. VEGF/VPF and FVIIIRAg immunohistochemistry and endothelial specific lectin (UEA1) binding studies, indicate that the elevation of VEGF protein expression initially occurred in both blood vessels and non-endothelial cells. After 15 days of E2 exposure, VEGF/VPF protein expression, in the non-endothelial cell population, sharply declined and was restricted to the blood vessels. The function of non-endothelial-derived VEGF is not clear. Furthermore, immunohistochemical studies demonstrated that VEGFR-2 (flk-1/KDR), expression was elevated significantly in the endothelial cells of microblood vessels after 7 days of E2 exposure. These findings suggest that over expression of VEGF and its receptor (VEGFR-2) may play an important role in the initial step of the regulation of estrogen induced tumor angiogenesis in the rat pituitary.

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21: EMBO J. 1997 Jul 1;16(13):3898-911.

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Proteolytic processing regulates receptor specificity and activity of VEGF-C.

Joukov V, Sorsa T, Kumar V, Jeltsch M, Claesson-Welsh L, Cao Y, Saksela O, Kalkkinen N, Alitalo K.

Molecular/Cancer Biology Laboratory, University of Helsinki, Finland.

The recently identified vascular endothelial growth factor C (VEGF-C) belongs to the platelet-derived growth factor (PDGF)/VEGF family of growth factors and is a ligand for the endothelial-specific receptor tyrosine kinases VEGFR-3 and VEGFR-2. The VEGF homology

domain spans only about one-third of the cysteine-rich VEGF-C precursor. Here we have analysed the role of post-translational processing in VEGF-C secretion and function, as well as the structure of the mature VEGF-C. The stepwise proteolytic processing of VEGF-C generated several VEGF-C forms with increased activity towards VEGFR-3, but only the fully processed VEGF-C could activate VEGFR-2. Recombinant 'mature' VEGF-C made in yeast bound VEGFR-3 (K[D] = 135 pM) and VEGFR-2 (K[D] = 410 pM) and activated these receptors. Like VEGF, mature VEGF-C increased vascular permeability, as well as the migration and proliferation of endothelial cells. Unlike other members of the PDGF/VEGF family, mature VEGF-C formed mostly non-covalent homodimers. These data implicate proteolytic processing as a regulator of VEGF-C activity, and reveal novel structure-function relationships in the PDGF/VEGF family.

Publication Types:
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☐ **22:** Dev Biol. 1997 Aug 1;188(1):96-109.

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ELSEVIER FULL TEXT ARTICLE

VEGF and VEGF-C: specific induction of angiogenesis and lymphangiogenesis in the differentiated avian chorioallantoic membrane.

Oh SJ, Jeltsch MM, Birkenhäger R, McCarthy JE, Weich HA, Christ B, Alitalo K, Wilting J.

Anatomisches Institut II, Albert-Ludwigs-Universität Freiburg, Albertstrasse 17, Freiburg, D-79104, Germany.

The lymphangiogenic potency of endothelial growth factors has not been studied to date. This is partially due to the lack of in vivo lymphangiogenesis assays. We have studied the lymphatics of differentiated avian chorioallantoic membrane (CAM) using microinjection of Mercox resin, semi- and ultrathin sectioning, immunohistochemical detection of fibronectin and alpha-smooth muscle actin, and in situ hybridization with VEGFR-2 and VEGFR-3 probes. CAM is drained by lymphatic vessels which are arranged in a regular pattern. Arterioles and arteries are accompanied by a pair of interconnected lymphatics and form a plexus around bigger arteries. Veins are also associated with lymphatics, particularly larger veins, which are surrounded by a lymphatic plexus. The lymphatics are characterized by an extremely thin endothelial lining, pores, and the absence of a basal lamina. Patches of the extracellular matrix can be

stained with an antibody against fibronectin. Lymphatic endothelial cells of differentiated CAM show ultrastructural features of this cell type. CAM lymphatics do not possess mediae. In contrast, the lymphatic trunks of the umbilical stalk are invested by a single but discontinuous layer of smooth muscle cells. CAM lymphatics express VEGFR-2 and VEGFR-3. Both the regular pattern and the typical structure of these lymphatics suggest that CAM is a suitable site to study the in vivo effects of potential lymphangiogenic factors. We have studied the effects of VEGF homo- and heterodimers, VEGF/PlGF heterodimers, and PlGF and VEGF-C homodimers on Day 13 CAM. All the growth factors containing at least one VEGF chain are angiogenic but do not induce lymphangiogenesis. PlGF-1 and PlGF-2 are neither angiogenic nor lymphangiogenic. VEGF-C is the first lymphangiogenic factor and seems to be highly chemoattractive for lymphatic endothelial cells. It induces proliferation of lymphatic endothelial cells and development of new lymphatic sinuses which are directed immediately beneath the chorionic epithelium. Our studies show that VEGF and VEGF-C are specific angiogenic and lymphangiogenic growth factors, respectively.

Publication Types:
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PMID: 9245515 [PubMed - indexed for MEDLINE]

23: Dev Dyn. 1997 Sep;210(1):66-77.

Related Articles, Links

Neovascularization of the Xenopus embryo.

Cleaver O, Tonissen KF, Saha MS, Krieg PA.

Institute for Cellular and Molecular Biology, University of Texas at Austin 78712, USA.

The receptor tyrosine kinase, Flk-1 or VEGFR-2, and its ligand, vascular endothelial growth factor (VEGF) are required for the development of the embryonic vasculature. Targeted disruption of either gene in mice results in the failure of vascular system formation. The Xenopus homologues of flk-1 and VEGF have been cloned and their expression has been examined throughout early embryonic development. These studies indicate that flk-1 is expressed in groups of endothelial precursor cells which will form the major blood vessels of the embryo, including the posterior cardinal veins, the dorsal aorta, the vitelline veins, and the endocardium. VEGF expression is found in tissues adjacent to the mesenchyme containing the flk-1-expressing endothelial precursors. Expression of both flk-1 and VEGF is transient, appearing as the primary vascular plexus is forming and declining steadily after the onset of functional embryonic circulation. After

establishment of the primary vascular structures, flk-1 expression is also observed in the intersegmental veins which form by an angiogenic mechanism. Overall, these results support a role for VEGF/flk-1 signaling in both vasculogenesis and angiogenesis in the Xenopus embryo. When VEGF is expressed ectopically in Xenopus embryos by microinjection of either plasmid DNA or synthetic mRNA, large, disorganized vascular structures are produced. This result indicates that ectopic VEGF is capable of altering the architecture of the developing vascular network.

Publication Types:
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24: J Cell Sci. 1997 Sep;110 (Pt 18):2293-302.

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Vascular endothelial growth factor-induced in vitro angiogenesis and plasminogen activator expression are dependent on endogenous basic fibroblast growth factor.

Mandriota SJ, Pepper MS.

Department of Morphology, University Medical Center, Geneva, Switzerland.

Induction of in vitro angiogenesis and upregulation of urokinase- and tissue type-plasminogen activator (uPA, tPA) expression are two hallmarks of vascular endothelial growth factor (VEGF) activity on cultured endothelial cells. We report here that neutralizing antibodies to basic fibroblast growth factor (bFGF) inhibit VEGF-induced in vitro angiogenesis in bovine microvascular endothelial (BME) cells. Analysis of VEGF receptor-2 (VEGFR-2) expression revealed no alteration in VEGFR-2 mRNA or total protein in anti-bFGF antibody-treated BME or bovine aortic endothelial (BAE) cells. Ethidium bromide/agarose gel electrophoresis on the cytosolic fraction of BME cells revealed a basal level of fragmented DNA that was increased by anti-bFGF antibodies to an extent not exceeding that observed in parallel cultures incubated with concentrations of transforming growth factor-ss1 that increase VEGF-induced in vitro angiogenesis. In both BME and BAE cells, antibodies to bFGF also decreased basal levels of cell-associated uPA activity, and completely blocked the VEGF-mediated increase in uPA and tPA expression observed in parallel cultures incubated with VEGF alone. In contrast, PA inhibitor-1 expression was strongly upregulated in BME and BAE cells incubated with antibodies to bFGF, either alone or in combination with VEGF. These findings demonstrate that: (1) VEGF-induced in vitro

angiogenesis and PA expression are dependent on endogenous bFGF, (2) that this phenomenon is not mediated by a decrease in VEGFR-2 expression and that apoptosis does not necessarily correlate with inhibition of invasion, and (3) that inhibition of endogenous bFGF in VEGF-treated cells results in a net antiproteolytic (and possibly also anti-adherent) effect, which could account in part for the inhibitory effect of the anti-bFGF antibodies. These findings point to a novel and unsuspected role for endogenous bFGF in regulating VEGF-induced in vitro angiogenesis.

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25: Nat Med. 1997 Nov;3(11):1222-7.

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Halting angiogenesis suppresses carcinoma cell invasion.

Skobe M, Rockwell P, Goldstein N, Vosseler S, Fusenig NE.

Division of Carcinogenesis and Differentiation, German Cancer Research Center (DKFZ), Heidelberg.

The importance of angiogenesis in malignant tumor growth has been interpreted mainly in terms of oxygen and nutrient supply. Here we demonstrate its fundamental role for tumor invasion of malignant human keratinocytes in surface transplants on nude mice. Distinct patterns of angiogenesis and vascular endothelial growth factor receptor-2 (VEGFR-2) expression allowed us to distinguish between benign and malignant cells. Functional inactivation of VEGF-R2 by a blocking antibody disrupted ongoing angiogenesis and prevented invasion of malignant cells, without reducing tumor cell proliferation. The reversion of a malignant into a benign phenotype by halting angiogenesis demonstrates a significant function of vascular endothelium for tumor invasion.

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PMID: 9359696 [PubMed - indexed for MEDLINE]

26: J Biol Chem. 1998 Mar 20;273(12):6599-602.

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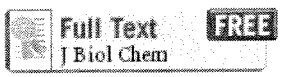

A recombinant mutant vascular endothelial growth factor-C that has lost vascular endothelial growth factor receptor-2 binding, activation, and vascular permeability activities.

Joukov V, Kumar V, Sorsa T, Arighi E, Weich H, Saksela O, Alitalo K.

Molecular/Cancer Biology Laboratory, Haartman Institute, PL 21 Haartmaninkatu 3, University of Helsinki, 00014 Helsinki, Finland.

The vascular endothelial growth factor (VEGF) and the VEGF-C promote growth of blood vessels and lymphatic vessels, respectively. VEGF activates the endothelial VEGF receptors (VEGFR) 1 and 2, and VEGF-C activates VEGFR-3 and VEGFR-2. Both VEGF and VEGF-C are also potent vascular permeability factors. Here we have analyzed the receptor binding and activating properties of several cysteine mutants of VEGF-C including those (Cys156 and Cys165), which in other platelet-derived growth factor/VEGF family members mediate interchain disulfide bonding. Surprisingly, we found that the recombinant mature VEGF-C in which Cys156 was replaced by a Ser residue is a selective agonist of VEGFR-3. This mutant, designated DeltaNDeltaC156S, binds and activates VEGFR-3 but neither binds VEGFR-2 nor activates its autophosphorylation or downstream signaling to the ERK/MAPK pathway. Unlike VEGF-C, DeltaNDeltaC156S neither induces vascular permeability in vivo nor stimulates migration of bovine capillary endothelial cells in culture. These data point out the critical role of VEGFR-2-mediated signal transduction for the vascular permeability activity of VEGF-C and strongly suggest that the redundant biological effects of VEGF and VEGF-C depend on binding and activation of VEGFR-2. The DeltaNDeltaC156S mutant may provide a valuable tool for the analysis of VEGF-C effects mediated selectively via VEGFR-3. The ability of DeltaNDeltaC156S to form homodimers also emphasizes differences in the structural requirements for VEGF and VEGF-C dimerization.

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27: J Cell Sci. 1998 Jul;111 (Pt 13):1853-65.

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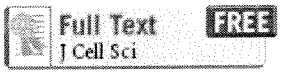

Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells.

Esser S, Lampugnani MG, Corada M, Dejana E, Risau W.

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Interendothelial junctions play an important role in the regulation of endothelial functions, such as vasculogenesis, angiogenesis, and vascular permeability. In this paper we show that vascular endothelial growth factor (VEGF), a potent inducer of new blood vessels and vascular permeability in vivo, stimulated the migration of endothelial cells after artificial monolayer wounding and induced an increase in paracellular permeability of human umbilical vein endothelial cells (HUVECs). Furthermore, VEGF increased phosphotyrosine labeling at cell-cell contacts. Biochemical analyses revealed a strong induction of VEGF-receptor-2 (flk-1/KDR) tyrosine-autophosphorylation by VEGF which was maximal after 5 minutes and was followed by receptor downregulation. 15 minutes to 1 hour after VEGF stimulation the endothelial adherens junction components VE-cadherin, beta-catenin, plakoglobin, and p120 were maximally phosphorylated on tyrosine, while alpha-catenin was not modified. PECAM-1/CD31, another cell-cell junctional adhesive molecule, was tyrosine phosphorylated with similar kinetics in response to VEGF. In contrast, activation of VEGF-receptor-1 (Flt-1) by its specific ligand placenta growth factor (PlGF) had no effect on the tyrosine phosphorylation of cadherins and catenins. Despite the rapid and transient receptor activation and the subsequent tyrosine phosphorylation of adherens junction proteins the cadherin complex remained stable and associated with junctions. Our results demonstrate that the endothelial adherens junction is a downstream target of VEGFR-2 signaling and suggest that tyrosine phosphorylation of its components may be involved in the the loosening of cell-cell contacts in established vessels to modulate transendothelial permeability and to allow sprouting and cell migration during angiogenesis.

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☐ **28:** Exp Cell Res. 1998 Jun 15;241(2):414-25.

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ELSEVIER FULL-TEXT ARTICLE

Regulation of vascular endothelial growth factor receptor-2 (Flk-1) expression in vascular endothelial cells.

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We have previously reported the existence of a synergistic interaction

between vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in the induction of angiogenesis in vitro. Here we demonstrate that bFGF increases VEGF receptor-2 (VEGFR-2/Flk-1) expression: mRNA levels were increased by 4.5- to 8.0-fold and total protein by 2.0- to 3.5-fold, in bovine microvascular endothelial (BME), aortic endothelial (BAE), and transformed fetal aortic (GM7373) endothelial cells. VEGF itself did not affect VEGFR-2 expression, and neither bFGF nor VEGF altered expression of FGF receptor-1. We also show that synergism occurs at the level of proliferation when this is measured in a three-dimensional but not in a conventional two-dimensional assay. Differences in the level of VEGFR-2 expression were also observed when cells were grown on or within collagen gels under different conditions: mRNA levels were lowest under sparse conditions, increased 20- to 26-fold at confluence, and increased even further (57-fold) when cells were cultured in suspension in three-dimensional collagen gels. Finally, a synergistic increase was seen in the level of expression of urokinase and urokinase receptor mRNAs when cells were exposed to bFGF and VEGF for 4 days. These findings demonstrate that the level of VEGFR-2 expression can be modulated by environmental factors including cytokines and the geometry of the culture conditions and provide some insight into the mechanisms of synergism between bFGF and VEGF in the induction of angiogenesis in vitro. Copyright 1998 Academic Press.

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29: J Biol Chem. 1998 Aug 21;273(34):22128-35.

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Tumor necrosis factor-alpha regulates expression of vascular endothelial growth factor receptor-2 and of its co-receptor neuropilin-1 in human vascular endothelial cells.

Giraudo E, Primo L, Audero E, Gerber HP, Koolwijk P, Soker S, Klagsbrun M, Ferrara N, Bussolino F.

Vascular Biology Laboratory, Department of Genetics, Biology and Biochemistry, Medical School, University of Torino, Torino, 10126 Italy.

Tumor necrosis factor-alpha (TNF-alpha) modulates gene expression in endothelial cells and is angiogenic in vivo. TNF-alpha does not activate in vitro migration and proliferation of endothelium, and its angiogenic activity is elicited by synthesis of direct angiogenic inducers or of proteases. Here, we show that TNF-alpha up-regulates

in a dose- and time-dependent manner the expression and the function of vascular endothelial growth factor receptor-2 (VEGFR-2) as well as the expression of its co-receptor neuropilin-1 in human endothelium. As inferred by nuclear run-on assay and transient expression of VEGFR-2 promoter-based reporter gene construct, the cytokine increased the transcription of the VEGFR-2 gene. Mithramycin, an inhibitor of binding of nuclear transcription factor Sp1 to the promoter consensus sequence, blocked activation of VEGFR-2, suggesting that the up-regulation of the receptor required Sp1 binding sites. TNF-alpha increased the cellular amounts of VEGFR-2 protein and tripled the high affinity 125I-VEGF-A165 capacity without affecting the Kd of ligand-receptor interaction. As a consequence, TNF-alpha enhanced the migration and the wound healing triggered by VEGF-A165. Since VEGFR-2 mediates angiogenic signals in endothelium, our data indicate that its up-regulation is another mechanism by which TNF-alpha is angiogenic and may provide insight into the mechanism of neovascularization as occurs in TNF-alpha-mediated pathological settings.

Publication Types:
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30: Am J Pathol. 1998 Aug;153(2):381-94.

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Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia.

Witzenbichler B, Asahara T, Murohara T, Silver M, Spyridopoulos I, Magner M, Principe N, Kearney M, Hu JS, Isner JM.

Department of Medicine, St. Elizabeth's Medical Center of Boston, Tufts University School of Medicine, Massachusetts 02135, USA.

Recently, vascular endothelial growth factor-C (VEGF-C or VEGF-2) was described as a specific ligand for the endothelial receptor tyrosine kinases VEGFR-2 and VEGFR-3. In vivo data, limited to constitutive overexpression in transgenic mice, have been interpreted as evidence that the growth-promoting effects of VEGF-C are restricted to development of the lymphatic vasculature. The current studies were designed to test the hypothesis that constitutive expression of VEGF-C in adult animals promotes angiogenesis. In vitro, VEGF-C exhibited a dose-dependent mitogenic and chemotactic effect on endothelial cells, particularly for microvascular endothelial cells (72% and 95% potency, respectively, compared with VEGF-A/VEGF-1). VEGF-C

stimulated release of nitric oxide from endothelial cells and increased vascular permeability in the Miles assay; the latter effect was attenuated by pretreatment with the nitric oxide synthase inhibitor N (omega)-nitro-L-arginine methyl ester. Both VEGFR-2 and VEGFR-3 receptors were shown to be expressed in human saphenous vein and internal mammary artery. The potential for VEGF-C to promote angiogenesis in vivo was then tested in a rabbit ischemic hindlimb model. Ten days after ligation of the external iliac artery, VEGF-C was administered as naked plasmid DNA (pcVEGF-C; 500 microg) from the polymer coating of an angioplasty balloon (n = 8 each) or as recombinant human protein (rhVEGF-C; 500 microg) by direct intra-arterial infusion. Physiological and anatomical assessments of angiogenesis 30 days later showed evidence of therapeutic angiogenesis for both pcVEGF-C and rhVEGF-C. Hindlimb blood pressure ratio (ischemic/normal) after pcVEGF-C increased to 0.83 +/- 0.03 after pcVEGF-C versus 0.59 +/- 0.04 ($P < 0.005$) in pGSVLacZ controls and to 0.76 +/- 0.04 after rhVEGF-C versus 0.58 +/- 0.03 ($P < 0.01$) in control rabbits receiving rabbit serum albumin. Doppler-derived iliac flow reserve was 2.7 +/- 0.1 versus 2.0 +/- 0.2 ($P < 0.05$) for pcVEGF-C versus LacZ controls and 2.9 +/- 0.3 versus 2.1 +/- 0.2 ($P < 0.05$) for rhVEGF-C versus albumin controls. Neovascularity was documented by angiography in vivo (angiographic scores: 0.85 +/- 0.05 versus 0.51 +/- 0.02 ($P < 0.001$) for plasmid DNA and 0.74 +/- 0.08 versus 0.53 +/- 0.03 ($P < 0.05$) for protein), and capillary density (per mm2) was measured at necropsy (252 +/- 12 versus 183 +/- 10 ($P < 0.005$) for plasmid DNA and 229 +/- 20 versus 164 +/- 20 ($P < 0.05$) for protein). In contrast to the results of gene targeting experiments, constitutive expression of VEGF-C in adult animals promotes angiogenesis in the setting of limb ischemia. VEGF-C and its receptors thus constitute an apparently redundant pathway for postnatal angiogenesis and may represent an alternative to VEGF-A for strategies of therapeutic angiogenesis in patients with limb and/or myocardial ischemia.

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